

In the specification:

Please amend Figure 8 as described on the enclosed annotated version of Figure 8. A clean copy of Figure 8 is also enclosed herewith.

Please amend Table II at pages 11-12 of the specification as follows:

Table II

Serpin	Accession Number	RSL sequence
PI or AAT, A1AT_HUMAN ALPHA-1-ANTITRYPSIN PRECURSOR (ALPHA-1 PROTEASE INHIBITOR) (ALPHA-1- ANTIPROTEINASE)	sp P01009	GTEAAGAMFLEAIPMSIPPE (SEQ ID NO: 77)
PIL or ATR, A1AU_HUMAN ALPHA-1-ANTITRYPSIN-RELATED PROTEIN PRECURSOR	sp P20848	GTEATGAPHLEEKAWSKYQT (SEQ ID NO: 78)
PLI OR AAP, A2AP_HUMAN ALPHA-2-ANTIPLASMIN PRECURSOR (ALPHA-2-PLASMIN INHIBITOR) (ALPHA-2-PI) (ALPHA-2-AP)	sp P08697	GVEAAAATSIAIMSRLMSLSSF (SEQ ID NO: 79)
AACT, AACT_HUMAN ALPHA-1-ANTICHYMO TRYPSIN PRECURSOR (ACT)	sp P01011	GTEASAATAVKITLLSALVE (SEQ ID NO: 80)
AT3, ANT3_HUMAN ANTITHROMBIN-III PRECURSOR (ATIII)	sp P01008	GSEAAA STAVVIAGRSLNPN (SEQ ID NO: 81)
PI10, BOMA_HUMAN BOMAPIN (PROTEASE INHIBITOR 10)	sp P48595	GTEAAAGSGSEIDIRIRVPS (SEQ ID NO: 82)
CBP2, CBP2_HUMAN COLLAGEN-BINDING PROTEIN 2 PRECURSOR (COLLIGIN 2)	sp P50454	GNPFDQDIYGREELRSPKLF (SEQ ID NO: 83)
PI7 or PN1, GDN_HUMAN GLIA DERIVED NEXIN PRECURSOR (GDN) (PROTEASE NEXIN I) (PN-1) (PROTEASE INHIBITOR 7)	sp P07093	GTKASAATTAILIARISSPPW (SEQ ID NO: 84)
HCF2, HEP2_HUMAN HEPARIN COFACTOR II PRECURSOR (HC-II) (PROTEASE INHIBITOR LEUSERPIN 2) (HLS2)	sp P05546	GTQATTVTVGFMPLSTQVR (SEQ ID NO: 85)
C1NH or C1IN, IC1_HUMAN PLASMA PROTEASE C1 INHIBITOR PRECURSOR (C1 INH)	sp P05155	GVEAAAASAISVARTLLVFE (SEQ ID NO: 86)
ELANH2 or PI2, ILEU_HUMAN LEUKOCYTE ELASTASE INHIBITOR (LEI) (MONOCYTE/NEUTROPHIL ELASTASE INHIBITOR) (M/NEI) (EI)	sp P30740	GTEAAAATAGIATFCMLMPE (SEQ ID NO: 87)
PCI or PLANH3 or PROCI, IPSP_HUMAN PLASMA SERINE PROTEASE INHIBITOR PRECURSOR (PCI) (PROTEIN C INHIBITOR) (PLASMINOGEN ACTIVATOR INHIBITOR-3) (PAI3)	sp P05154	GTRAAAATGTIFTFRSARLN (SEQ ID NO: 88)
PI4 or KST, KAIN_HUMAN KALLISTATIN PRECURSOR (KALLIKREIN INHIBITOR) (PROTEASE INHIBITOR 4)	sp P29622	GTEAAAATTFAIKFFSAQTN (SEQ ID NO: 89)
PI5, MASP_HUMAN MASPIN PRECURSOR (PROTEASE INHIBITOR 5)	sp P36952	GGDSIEVPGARILQHKDELN (SEQ ID NO: 90)
PI12, NEUS_HUMAN NEUROSERPIN PRECURSOR (PROTEASE INHIBITOR 12)	sp Q99574	GSEAAAVSGMIAISRMADVLY (SEQ ID NO: 91)
PAI1 or PLANH1, sp P05121 PAI1_HUMAN PLASMINOGEN ACTIVATOR INHIBITOR-1 PRECURSOR, ENDOTHELIAL (PAI-1)	sp P05121	GTVASSSSTAVIVSARMAPEE (SEQ ID NO: 92)
PAI2 or PLANH2, PAI2_HUMAN PLASMINOGEN ACTIVATOR INHIBITOR-2, PLACENTAL (PAI-2) (MONOCYTE ARG- SERPIN) (UROKINASE INHIBITOR)	sp P05120	GTEAAAGTGGVMIGRTGHGG (SEQ ID NO: 93)

PEDF, PEDF_HUMAN PIGMENT EPITHELIUM-DERIVED FACTOR PRECURSOR (PEDF) (EPC-1)	sp P36955	GAGTTPSPLQPAHLTFPLD <u>(SEQ ID NO: 94)</u>
PI6 or PTI, PTI6_HUMAN PLACENTAL THROMBIN INHIBITOR (CYTOPLASMIC ANTIPROTEINASE) (CAP) (PROTEASE INHIBITOR 6)	sp P35237	GTEAAAATAAIIIMMRCARFV <u>(SEQ ID NO: 95)</u>
PI8, PTI8_HUMAN CYTOPLASMIC ANTIPROTEINASE 2 (CAP2) (CAP-2) (PROTEASE INHIBITOR 8)	sp P50452	GTEAAAATAVVRNSRCSRME <u>(SEQ ID NO: 96)</u>
PI9, PTI9_HUMAN CYTOPLASMIC ANTIPROTEINASE 3 (CAP3) (CAP-3) (PROTEASE INHIBITOR 9)	sp P50453	GTEAAAASSCFVVAECCMES <u>(SEQ ID NO: 97)</u>
SCCA1, SCC1_HUMAN SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1) (PROTEIN T4-A)	sp P29508	GAEAAAATAVVGFGSSPAST <u>(SEQ ID NO: 98)</u>
SCCA2, SCC2_HUMAN SQUAMOUS CELL CARCINOMA ANTIGEN 2 (SCCA-2) (LEUPIN)	sp P48594	GVEAAAATAVVVVELSSPST <u>(SEQ ID NO: 99)</u>
TBG, THBG_HUMAN THYROXINE-BINDING GLOBULIN PRECURSOR (T4-BINDING GLOBULIN)	sp P05543	GTEAAAVPEVELSDQPENTF <u>(SEQ ID NO: 100)</u>
MEGSIN	gi 4505149 ref NP_003775.1	GTEATAATGSNIVEKQLPQS <u>(SEQ ID NO: 101)</u>
PI14, pancpin, TSA2004	gi 3724282 dbj BAA33766.1	GSEAAATSTGIHIPVIMSLAQ <u>(SEQ ID NO: 102)</u>

Please amend the paragraph at page 26, lines 21-33 as follows:

---hK2 and hK3 (PSA) were purified from human semen as previously described (Frenette G, Gervais Y, Tremblay RR, Dube JY. **1998** "Contamination of purified prostate-specific antigen preparations by kallikrein hK2" *J Urol* 159, 1375-8), anti-hK2 and anti-PSA monoclonal antibodies were a gift from Professor RR Tremblay, Laval University, Canada. Human chymotrypsin (Chtr), urokinase plasminogen activator (uPA), human kallikrein hK1, human plasma kallikrein (PK), human neutrophil elastase (HNE) and commercial ACT (human plasma α 1-antichymotrypsin) were purchased from Calbiochem. Z-Phe-Arg-AMC, Suc-Ala-Ala-Pro-Phe-AMC (SEQ ID NO: 103), Z-Gly-Gly-Arg-AMC, MeOSuc-Ala-Ala-Pro-Val-AMC (SEQ ID NO: 104) were purchased from Calbiochem. CFP-TFRSA-YFP (SEQ ID NO: 130) fluorescent substrate was developed as previously described (Mahajan NP et al. **1999** "Novel mutant green fluorescent protein protease substrates reveal the activation of specific caspases during apoptosis" *Chem Biol* 6, 401-9). The cDNA for human α 1-antichymotrypsin (ACT) was a generous gift from Dr. Harvey Rubin (University of Pennsylvania).---

Please amend the paragraph at page 27, lines 3-9 as follows:

---Following the subcloning of ACT cDNA into pQE-9 expression vector (Qiagen, Germany, figure 9) and the introduction of an His₆ tag at the N-terminal of rACT_{WT}, two restriction sites *Sac II* and *MluI*, were incorporated 18 bp upstream and 18 bp downstream of P1 codon in RSL domain respectively. These sites were created by silent mutation using oligonucleotides 5'- GTGATTTCACCGCGTGGCAGCAG-3' (SEQ ID NO: 105) for *Sac II* and 5'- GCACAATGGTACGCGTC TCCACTAATG-3' (SEQ ID NO: 106) for *Mlu I* site and following the quickchange mutagenesis protocol supplied by Stratagene.---

Please amend the paragraph at page 27, lines 12-21 as follows:

---Substrate phage libraries were generated using a modified pH0508b phagemid (*Lowman et al. 1991* "Selecting high-affinity binding proteins by monovalent phage display" *Biochemistry* 12, 10832-8). The construction consists of a His₆ tag at either end of a Gly-Gly-Gly-Ser-(SEQ ID NO: 107) repeat-rich region that precedes the carboxyl-terminal domain (codons 249-406) of the M13 gene III. The random pentamers were generated by PCR extension of the template oligonucleotides with appropriate restriction sites positioned on both side of the degenerate codons: 5'TGAGCTAGTCTAGATAGGTGGCGGTNNNSNNNSNNNSGGTC GACGTGGTCATAGCAGTCGCTGCA-3' (SEQ ID NO: 108) (where N is any nucleotide and S is either G or C) using 5' biotinylated primers corresponding to the flanking regions: 5'TGAGCTAGTCTAGATAGGTG-3' (SEQ ID NO: 109) and 5'-TGCAGCGACTGCTATGA-3'. (SEQ ID NO: 110).---

Please amend the paragraph at page 28, line 16 to page 29, line 17 as follows:

---Six variants, which correspond to a change in the reactive site loop in positions between P3 and P3' (see Table IV below), were generated by PCR extension of the template oligonucleotides:

rACT_{8.20}, 5'-TACCGCGGTAAAATCACCCTCCGTTCTGAGCAGTGGA
GACGCGT GA-3' (SEQ ID NO: 111);

rACT_{6.3}, 5'-TACCGCGGTCAAAATCACCCAGGAGGTCTATCGATGT

GGAGACGCGTGA-3'(SEQ ID NO: 112);

rACT_{8.3}, 5'-TACCGCGGTCAAAATCAGGGGGAGATCTGAGTTAGTG

GAGACGCGTGA-3'(SEQ ID NO: 113);

rACT_{6.7}, 5'-TACCGCGGTCAAAATCAAGCTTAGAACACATTAG

TGGAGACCGCTGA-3'(SEQ ID NO: 114);

rACT_{6.1}, 5'-TACCGCGGTCAAAATCATGACAAGATCTAACTTAGT

GGAGACGCGTGA-3'(SEQ ID NO: 115);

rACT_{5.18}, 5'-TACCGCGGTCAAAATCACCGAGCGTGTCTGCCCGTG

GAGACGCGTGA-3'(SEQ ID NO: 116)

(where underlined sequences encode new cleavage sites in the reactive site loop), using primers corresponding to the flanking regions : 5'-TACCGCGGTCAAAATC-3' (SEQ ID NO: 117) and 5'-TCACGCGTGTCCAC-3' (SEQ ID NO: 118). PCR products were digested with *Sac II* and *Mlu I* restriction enzymes and then subcloned into digested rACT_{WT} construct. Recombinant serpins were produced in TG1 *E. coli* strain. Cells were grown at 37°C in 2 x TY media (16g tryptone, 10g yeast extract, 5g NaCl per L) containing 100µg/ml ampicillin to A₆₀₀=0.5.

Isopropylthio-β-galactoside (IPTG) was then added to a final concentration of 0.5mM allowing the expression of recombinant serpins for 16h at 16°C. The cells from 100ml of culture were harvested by centrifugation, resuspended in cold PBS and then passed through a french press to recover the total soluble cytoplasmic proteins. Cell debris were removed by centrifugation and Ni²⁺-nitrotriacetic affinity agarose beads were added to supernatant for 90 min at 4°C to bind recombinant serpins. The resin was subsequently washed with 50mM Tris pH 8.0, 500mM NaCl, 25mM Imidazole and the bound proteins were eluted for 10min with 50mM Tris pH 8.0, 500mM NaCl and 150mM Imidazole. Once purification was completed, rACT were dialysed against 50mM Tris pH 8.0, 500mM NaCl, 0,05 % Triton X-100 for 16h at 4°C. The protein concentration was determined for each purification by Bradford assay and normalized by densitometry of Coomassie Blue-stained SDS-PAGE gels (*Laemmli UK. 1970 “Cleavage of structural proteins during the assembly of the head of bacteriophage T4” Nature 227, 680-5*).---

Please amend Table IV at page 29 as follows:

Alignment of RSL (Reactive Serpin Loop) of recombinant serpin $\alpha 1$ -antichymotrypsin (ACT) and its variants.

<i>Serpin</i>	<i>Selected^a Substrate Peptide</i>	<i>P6</i>	<i>P5</i>	<i>P4</i>	<i>P3</i>	<i>P2</i>	<i>PI</i>	<i>P'1</i>	<i>P'2</i>	<i>P'3</i>	<i>P'4</i>	<i>P'5</i>	<i>P'6</i>	<i>SEQ. NO</i>
rACT_{WT}		V	K	I	T	L	<u>L</u> *	S	A	L	V	E	T	<u>119</u>
rACT_{8.20}	LR↓SRA	V	K	I	T	<u>L</u>	<u>R</u> *	S	<u>R</u>	<u>A</u>	V	E	T	<u>120</u>
rACT_{6.2}	RR↓SID	V	K	I	T	<u>R</u>	<u>R</u> *	S	<u>I</u>	<u>D</u>	V	E	T	<u>121</u>
rACT_{8.3}	RGR↓SE	V	K	I	<u>R</u>	<u>G</u>	<u>R</u> *	<u>S</u>	<u>E</u>	L	V	E	T	<u>122</u>
rACT_{6.7}	KLR↓TT	V	K	I	<u>K</u>	<u>L</u>	<u>R</u> *	<u>T</u>	<u>T</u>	L	V	E	T	<u>123</u>
rACT_{6.1}	MTR↓SN	V	K	I	<u>M</u>	<u>T</u>	<u>R</u> *	<u>S</u>	<u>N</u>	<u>A</u>	V	E	T	<u>124</u>
ACT_{5.18}	ER↓VSP	V	K	I	T	<u>E</u>	<u>R</u> *	<u>V</u>	<u>S</u>	<u>P</u>	V	E	T	<u>125</u>

^a Substrate peptides selected by kallikrein hK2 using a phage-displayed random pentapeptide library . Plain type residues are common to rACT_{WT}, bold and underlined residues correspond to substrate peptides relocated in RSL of ACT variants. The scissile bond by hK2 in substrate peptides is designated by ↓ and putative cleavage site in serpins is marked by asterisks between the P1-P1' residues.

Please amend Table VII at page 36 as follows:

TABLE VII

Alignment of RSL (Reactive Serpin Loop) of recombinant serpins ACT, PCI and ACT_{PCI}.

<i>RSL sequences</i>														
<i>Serpin</i>	<i>P6</i>	<i>P5</i>	<i>P4</i>	<i>P3</i>	<i>P2</i>	<i>PI</i>	<i>P'1</i>	<i>P'2</i>	<i>P'3</i>	<i>P'4</i>	<i>P'5</i>	<i>P'6</i>		
rACT_{WT}	Amino acid sequence	V	K	I	T	L	<u>L</u>	S	A	L	V	E	T	
		<u>SEQ</u>	<u>ID</u>	<u>NO:</u>	<u>119</u>									
	DNA sequence (codon)	GTC	AAA	ATC	ACC	CTC	CTT	TCT	GCA	TTA	GTG	GAG	GTC	
		<u>SEQ</u>	<u>ID</u>	<u>NO:</u>	<u>126</u>									

rPCI_{WT}	Amino acid sequence	T <u>SEQ</u>	I <u>ID</u>	F <u>NO:</u>	T <u>127</u>	F	R	S	A	R	L	N	S
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rACT_{PCI}	(MD CI)	Amino acid sequence	V <u>SEQ</u>	K <u>ID</u>	I <u>NO:</u>	T <u>128</u>	F	R	S	A	L	V	E	T
		DNA sequence (codon)	GTC	AAA	ATC	ACC	TTT	AGA	TCT	GCA	TTA	GTG	GAG	GTC
			<u>SEQ</u>	<u>ID</u>	<u>NO:</u>	<u>129</u>								

Plain type residues are common to rACT_{WT}, bold and underlined residues correspond to substrate peptides relocated in RSL of ACT variants. The scissile bond in substrate peptides is designated by ↓ and putative cleavage site in serpins is marked by asterisks between the P1-P1' residues.